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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of:
MAGILL, *et al.*
Serial No.: 09/975,020
Filed: 12 October 2001

For: MICROFLUIDIZED LEISHMANIA LYSATE AND METHODS OF MAKING AND USING THEREOF

Art Unit: 1645
Examiner: Shahnan Shah, Khatol S
Atty. Dckt: 034047.013 (WRAIR 98-40/46)

APPELLANTS' BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Mail Stop: Appeal Briefs Patents

Dear Sir:

This is Appellants' Brief on Appeal in response to the Advisory Action mailed in the above-referenced case. The due date for filing this Appeal Brief was 24 September 2004, and a Petition for a Two-Month Extension of Time and the appropriate fee have been concurrently filed herewith in order to extend the due date to 25 October 2004.

1. REAL PARTY IN INTEREST

The present application is assigned to:

U.S. Army Medical Research and Materiel Command

2. RELATED APPEALS AND INTERFERENCES

To the best of the undersigned's knowledge, no other appeals or interferences will directly affect, will be directly affected by, or will have a bearing on the Board's Decision in this appeal.

3. STATUS OF CLAIMS

A statement of the status of all the claims, pending or cancelled, and identifying the claims appealed.

Claims 1-3, 5-10, 13-21, and 26-28 have been canceled.

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01 FC:1402 340.00 DA

10/29/2004 CNGUYEN 00000001 210380 09975020
01 FC:1402 340.00 DA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
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TRANSMITTAL OF APPELLANTS' APPEAL BRIEF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Mail Stop: Appeal Briefs Patents

Dear Sir:

Further to the Notice of Appeal filed 24 June 2004, enclosed please find Appellants' Brief on Appeal. Please charge **Deposit Account No. 210-380,** Attorney Docket No. **034047.013 (WRAIR 98-40/46)** in the amount of $340.00 for filing this brief in support of an appeal under 37 CFR 41.20(b)(2). A duplicate sheet is attached.

Also submitted herewith is a Petition for a Two-Month Extension of Time and the requisite fee.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. §1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 210-380,** Attorney Docket No. **034047.013 (WRAIR 98-40/46).**

Respectfully submitted,

Suzannah K. Sundby
Registration No. 43,172

Date: 25 October 2004
SMITH, GAMBRELL & RUSSELL, LLP
1850 M Street, N.W., Suite 800
Washington, D.C. 20036
Telephone: (202) 263-4332

Certificate Mailing or Transmission under 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being:

☒ deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Mail Stop: Appeal Briefs Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

☐ transmitted by facsimile on the date shown below to the United States Patent and Trademark Office at (703) 872-9306.

On 25 October 2004, by Suzannah K. Sundby

Signed:

Claims 4, 11-12, 22-25, and 29-31 remain pending in the application, and are appealed. These claims are found in the Claims Appendix.

4. STATUS OF AMENDMENTS

The amendment to the claims filed 25 November 2003 was entered. There are no outstanding unentered claim amendments. In the Advisory Action mailed 5 August 2004, the Examiner indicated that the affidavit of Dr. Jonathan B. Berman was not timely filed, but was considered in order to expedite prosecution.

5. SUMMARY OF CLAIMED SUBJECT MATTER

Claim 4 relates to a microfluidized lysate preparation that is free of dextran and is made by microfluidizing a slurry of at least one Leishmania parasite strain through a chamber and disrupting the leishmania parasite strain with a sudden release of pressure. *See* pages 16-17, paragraphs 62-63; page 7, paragraphs 29-30; and the claims as originally filed.

Claim 22 further limits the invention of claim 4 such that the preparation further comprises a pharmaceutically acceptable stabilizer and claim 23 indicates that the stabilizer is phenol. *See* page 5, paragraph 19; page 9, paragraph 36; page 12, paragraph 44; page 16, paragraph 60; page 17, paragraph 63, page 19, paragraph 71; and the claims as originally filed.

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Whether the Examiner committed reversible error in rejecting claims 4, 11, 12, 22-25, and 29-31 under 35 U.S.C. 102(b) as the Examiner deemed the claims were anticipated by Leishmania Research project DoD-8b (DoD-8b) or Stiteler et al. Production of Leishmania Skin Antigen Test GMP Protocol requirements 1 and 2, 1994 and 1995 (Stiteler I and Stiteler II, respectively).

7. ARGUMENT

A. The Examiner committed reversible error in rejecting claims 4, 11, 12, 22-25, and 29-31 under 35 U.S.C. 102(b) as being anticipated by DoD-8b or Stiteler I and Stiteler II.

The Cited Prior Art

For convenience, the following provides the substantive parts of the disclosures of the cited prior art which may be found as published in the Evidence Appendix attached hereto.

1. THE DISCLOSURE OF DOD-8B

> Synopsis: This study continues development of a skin test for leishmaniasis (like the skin test for tuberculosis) that would help diagnose this parasitic infection in Gulf War veterans and others who may have been exposed.
> Overall Project Objective: Develop an intradermal skin test for the screening of U.S. Service members who may have been exposed to leishmaniasis endemic areas.
> Status/Results to Date: As reported last year, the lyophilized LSTA was reformulated into a liquid product to avoid a suspected hypersensitivity to a component of the lyophilization buffer. A new IND for this reformulated Microfluidized-lysate (MFL)-LSTA was submitted to the FDA in 1999. A Phase I clinical trial was conducted in 15 healthy volunteers which demonstrated safety of the product by showing no significant local or systemic reactions to the product. Additionally, the product was administered in increasing dose and demonstrated that the skin test antigen had no significant local or systemic side effects when used at the planned maximal does. A RFP was released to identify a commercial manufacturer for the future licensure of the LSTA product. A contract was awarded and phase I/II dose ranging and potency trials are underway.
> Specific Aims: The goal is to identify a safe, potent, and non-sensitizing Leishmania Skin Test Antigen (LSTA); manufacture it under cGMP; obtain an IND for its use in phase I, II, and III clinical trials; and obtain ultimately a commercially available, FDA-licensed product.
> Methodology: Skin tests are widely accepted diagnostic interventions for diagnosis of prior infection with an infectious agent (e.g., tuberculosis). Currently there is no Leishmania skin test licensed for use in the USA. Once required phase I and phase II studies are completed in humans, studies could be performed in Gulf War veterans with confirmed and suspected leishmaniasis.

II. STITELER 1994

> Viscerotropic Leishmaniasis caused by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis in U.S. troops returning from Operation Desert Storm (ODS). The prevalence of *Leishmania* infection in ODS veterans is unknown. To determine the scope of infection in ODS veterans, a sensitive screening test is needed. One approach is to develop a *Leishmania* Skin Test (LST), which will meet FDA

requirements for safety and efficacy. The first step in the development of a safe LST is the production of LST antigen (LSTA) under the strict conditions of the FDA's Good Manufacturing Practices (GMP). Compliance with GMP in production of the LSTA should allow for the approval of Human Use studies with the LSTA by the FDA following their review of an Investigational New Drug (IND) Application. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of viscerotropic Leishmaniasis was chosen as the type strain of *L. tropica* and source of the LSTA. WR#1063 was cloned, characterized as *L. tropica* by isoenzyme analysis, and then expanded and cryo-preserved as a Master Seed Lot (MSL). One sample of the MSL was expanded under conditions of GMP in WRAIR's Pilot Bioproduction Facility to produce a Production Seed Lot (PSL). Individual samples of the PSL were expanded under GMP to produce Bulk Lot Productions (BLP) of whole promastigotes for use in development of LST protocols for both animal as well as Human Use studies.

III. STITELER 1995

Viscerotropic Leishmaniasis (VTL) resulting from infection by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis following isolations and characterization of the parasite from U.S. troops returning from Operation Desert Storm (ODS). The prevalence of VTL in ODS veterans is unknown. The USA/DoD decided to pursue the development of LSTA for use as such a diagnostic screening method to determine exposure of personnel to *L. tropica*. A soluble, lyophilized, Microfluidized lystate (MFL) LSTA was developed and produced in accordance with FDA guidelines for current GMP within WRAIR's Pilot Bioproduction Facility. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of VTL was chosen as the type strain and source of the MSL was then expanded (PSL),. Individual cryostocks of the PSL of WR#1063 promastigotes were grown, harvested, washed, and stored (BLP),. Various BLP processing experiments and animal testing of these LSRA preparations led to the current MFL-LSTA protocol. In brief, the BLP was thawed, microfluidized, centrifuged, the supernatant sterile filtered, the filtrate adjusted to dose, lyophilized as MFL-LSTA. Following required testing of the MFL-LSTA, an INDA was prepared for review by FDA. FDA's approval of human use will lead to Phase I/Phase II trials of the LSTA.

A1. Errors in the rejection

On page 2 in the Advisory Action mailed 5 August 2004, the Examiner simply, but erroneously, stated, "The prior art teaches the claimed product".

In order to anticipate, a reference must be enabling

The Examiner has repeatedly ignored and has failed to address Appellants' assertion that the cited prior art are nonenabling references and therefore can not be used to anticipate the claimed invention. Specifically, Appellants have repeatedly asserted that DoD-8b, Stiteler I, and

Stiteler II do not teach that the preparations MUST be free of dextran in each and every instance. Appellants also submitted the Affidavit of Dr. Berman who declares that DoD-8b, Stiteler I, and Stiteler II do not teach that the preparations MUST be free of dextran.

In the Advisory Action, the Examiner indicated that he considered the Affidavit of Dr. Berman. The Examiner stated that he:

> agrees with Dr. Berman in regard to the finding that Leishmania Research project DoD-8B does not recite that the Microfluidized –lysate preparation is free of dextran. But the prior art does not recite that the Microfluidized –lysate would contain dextran either.

The Examiner clearly disregards the declaration of one skilled in the art, Dr. Berman, that the cited prior art references do not contain enabling disclosures for microfluidized lysate preparations that are free of dextran. In the Berman Declaration, Dr. Berman declares that the cited prior art does not provide an enabling disclosure of the present invention as claimed. Specifically, Dr. Berman declares that he has read the cited prior art and does not understand the cited prior art as disclosing the preparations being *free of dextran* and *containing phenol*. Dr. Berman also declares that it would not be obvious to him to remove dextran from the formulations in order to prevent hypersensitivity. In order to anticipate, a reference must be enabling. Since the cited prior art do not provide an enabling disclosure of the present invention as claimed, the cited prior art do not anticipate the present invention. Clearly, the cited references do not contain enabling disclosures that teach microfluidized lysate preparations that are ALWAYS free of dextran.

The Examiner ignores the Berman Declaration providing that the cited references are nonenabling disclosures and asserts that Appellants must provide evidence that the microfluidized lysate preparations of the prior art references actually contain dextran. Appellants are unaware of a law or case setting forth such a requirement.

Nevertheless, Appellants have previously submitted that the first generation and second generation preparations are significantly different, i.e. the first generation contains dextran and the second generation (as presently claimed) does not contain dextran – which Appellants should know because the cited references are Appellants' own art. Ironically, the differences provided by the Appellants appear to be ignored by the Examiner.

To anticipate, a reference must teach each and every limitation

Appellants respectfully submit that the negative limitation "free of dextran" should be given the same consideration as a positive limitation. For a claim to a composition comprising ingredient A, ingredient B, and ingredient C, a prior art reference would anticipate the claim if the prior art reference disclosed a composition comprising ingredient A, ingredient B, and ingredient C. For a claim to a composition comprising ingredient A and ingredient B, but not ingredient C, the reference must teach a composition comprising ingredient A and ingredient B AND the ABSENCE of ingredient C in order to anticipate.

The cited references do not teach the limitation "free of dextran". Additionally, the cited references do not teach the presence of "phenol". Clearly, the cited references do not teach each and every limitation of the claimed invention.

A lyophilization buffer component suspected of causing hypersensitivity does not equal dextran

and

A liquid product does not equal phenol

In the Office action mailed 24 February 2004, the Examiner cites DoD-8b for holding that:

> In 1999 the second generation of the lysate was reformulated into a liquid product **(i.e. phenol)** to avoid a suspected hypersensitivity to a component of the lyophilization buffer **(i.e. dextran)**. See page 4 (emphasis added).

Appellants have read and re-read DoD-8b countless times and nowhere can Appellants find support for the Examiner's "**i.e.**" assertions. Numerous pharmaceutical products contain dextran. Many such pharmaceutical products containing dextran do not cause hypersensitivity reactions. Thus, one can not extrapolate from the cited prior art that the preparations must be free of dextran. Further, there are many solutions, solvents, buffers, and pharmaceutical carriers that are used to make liquid formulations. Phenol is just one of many. One can not simply assert that since a formulation is liquid, phenol must be an ingredient. This would be equivalent to asserting that all liquids contain phenol and therefore water contains phenol as water is a liquid.

Thus, it appears that the Examiner improperly used the Appellants' teachings in the specification to support the erroneous assertions and illogical reasoning that (1) the presence of phenol in a composition makes the composition a liquid product and (2) dextran is indeed the component that causes hypersensitivity. If the Examiner did not use the Appellants' own teachings in the specification, then the Examiner apparently made unsupportable conclusions that have no scientific foundation as the Examiner fails to provide any logical reasoning as to why a liquid product must always indicate the presence of phenol and components that cause hypersensitivity are always dextran.

The Examiner appears to have completely ignored Dr. Berman's declaration that:

> Simply reformulating a preparation in order to prevent hypersensitivity does not indicate that the preparation is free of dextran as there are many pharmaceutical preparations that contain dextran but do not cause hypersensitivity.

and

> The indication that a preparation is a liquid product does not indicate that the preparation contains phenol as there are numerous solutions, solvents, buffers, and pharmaceutical carriers that are used for liquid formulations.

It is completely wrong and improper to consider limitations NOT in the claims

The Examiner stated:

> Limitations such as use of the product in kits or pharmaceutical composition will be inherent in the teachings of Leishmania Research project DoD-8B.

There are no "use of" limitations in the claims. Instead, Appellants have repeatedly tried to explain that the limitations IN the claims to be considered and given patentable weight are "free of dextran" and "phenol". Appellants believe that the Examiner is confused by the explanation why the absence of dextran is significant and that such absence (and its significance) can not be elucidated from the cited prior art.

Nowhere do the cited prior art teach or suggest microfluidized lysate preparations that are ALWAYS free of dextran. The absence of dextran is important as a subject who had never been previously exposed to a Leishmania parasite, may exhibit a type I hypersensitivity reaction which may be incorrectly interpreted as a positive reaction indicating exposure to a Leishmania parasite. Nowhere do the cited prior art teach or suggest a microfluidized lysate preparation that is suitable for reliable assays, i.e. little to no false positives. Nowhere in the cited prior art is a

microfluidized Leishmania lysate preparation free of dextran disclosed or suggested. Nowhere in the cited prior art is a microfluidized Leishmania lysate preparation free of dextran and containing phenol disclosed or suggested.

A2. Specific limitations not described in the cited prior art

Claim 4

Claim 4 reads as follows:

> A microfluidized lysate preparation free of dextran made by microfluidizing a slurry of at least one *Leishmania* parasite strain through a chamber and disrupting the leishmania parasite strain with a sudden release of pressure..

The broadest claim, claim 4, is limited to microfluidized lysate preparations that are free of dextran. Nowhere do the cited prior art teach or suggest microfluidized lysate preparations that are free of dextran. A key word search and contextual interpretation of the words and phrases of the cited prior art do not expressly, explicitly, implicitly, or inherently teach or suggest that dextran is necessarily always present in the preparations. Likewise, the cited prior art do not explicitly or implicitly teach or suggest that dextran is necessarily always absent from the preparations.

Nowhere in the cited prior art references is a microfluidized lysate preparation free of dextran made by microfluidizing a slurry of at least one *Leishmania* parasite strain through a chamber and disrupting the leishmania parasite strain with a sudden release of pressure taught or suggested. Nowhere do the cited prior art references teach or suggest limitation – free of dextran. Nowhere do the cited prior art references teach or suggest microfluidizing a slurry of at least one Leishmania parasite strain by disrupting the leishmania parasite strain with a sudden release or pressure. Therefore, claim 4 and its dependent claims are novel over the prior art and the rejection under 35 U.S.C. 102(b) should properly be reversed.

Claim 23

Claim 23 is directed to the microfluidized lysate preparation of claim 4 and containing phenol.

Nowhere do the cited prior art teach or suggest microfluidized lysate preparations that are free of dextran and contain phenol. A key word search and contextual interpretation of the

words and phrases of the cited prior art do not expressly, explicitly, implicitly, or inherently teach or suggest that the preparations contain phenol and are dextran free. Therefore, claim 23 and its dependent claims are novel over the prior art and the rejection under 35 U.S.C. 102(b) should properly be reversed.

In summary,

1. In order to anticipate, a reference must be enabling.

2. To anticipate, a reference must teach each and every limitation.

3. A lyophilization buffer component suspected of causing hypersensitivity does not equal dextran.

4. A liquid product does not equal phenol.

5. It is completely wrong and improper to consider limitations NOT in the claims.

The cited prior art does not teach or suggest:

1. Microfluidized lysate preparations that are necessarily or always "free of dextran".

2. Microfluidized lysate preparations that "contain phenol".

Therefore, the prior art does not anticipate the claimed invention and the rejection under 35 U.S.C. 102(b) should properly be reversed.

For the reasons set forth above, Appellants respectfully submit that the rejections under 35 U.S.C. 102(b) of record are improper, and that these rejections of the claims are therefore overcome. Appellants therefore respectfully requests that these rejections of the Examiner be reversed.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. §1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 210-380,** Attorney Docket No. **034047.013 (WRAIR 98-40/46)**.

If any fees are due in connection with this Appeal Brief, please charge the fees to **Deposit Account No. 210-380,** Attorney Docket No. **034047.013 (WRAIR 98-40/46).**

Respectfully submitted,

Suzannah K. Sundby
Registration No. 43,172

Date: 25 October 2004
SMITH, GAMBRELL & RUSSELL, LLP
1850 M Street, N.W., Suite 800
Washington, D.C. 20036
Telephone: (202) 263-4332

Certificate Mailing or Transmission under 37 C.F.R. 1.8(a)

I hereby certify that this correspondence is being:

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☐ transmitted by facsimile on the date shown below to the United States Patent and Trademark Office at (703) 872-9306.

On 25 October 2004, by Suzannah K. Sundby

Signed:

8. CLAIMS APPENDIX

4. A microfluidized lysate preparation free of dextran made by microfluidizing a slurry of at least one *Leishmania* parasite strain through a chamber and disrupting the leishmania parasite strain with a sudden release of pressure.

11. A kit comprising the microfluidized lysate preparation of claim 4 and directions for determining whether a subject has been exposed to a *Leishmania* parasite or was afflicted with Leishmaniasis.

12. The kit of claim 11, wherein the *Leishmania* parasite strain is *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.*

22. The microfluidized lysate preparation of claim 4, and further comprising a pharmaceutically acceptable stabilizer.

23. The microfluidized lysate preparation of claim 22, wherein the pharmaceutically acceptable stabilizer is phenol.

24. The microfluidized lysate preparation of claim 22, wherein the composition is in the form of a liquid.

25. The microfluidized lysate preparation of claim 22, wherein the composition may be frozen or freeze-dried.

29. The microfluidized lysate preparation of claim 4, wherein the microfluidized lysate preparation is heat treated.

30. The microfluidized lysate preparation of claim 4, wherein the *Leishmania* parasite strain is *L. tropica, L. mexicana, L. guyanensis, L. braziliensis, L. major, L. donovani, L. chagasi, L. amazonensis, L. peruviana, L. panamensis, L. pifanoi, L. infantum,* or *L. aethiopica.*

31. An immunogenic composition comprising the microfluidized lysate preparation of claim 4.

9. EVIDENCE APPENDIX

9A. Berman Declaration

10. Related Proceedings Appendix

NA

Exhibit 9A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Magill, et al.	Group Art Unit: 1645
Serial No.: 09/975,020	Examiner: Shahnan Shah, Khatol S.
Filed: 12 October 2001	Atty Dkt No.: 034047.013US (WRAIR 98-40/46)
For: MICROFLUIDIZED LEISHMANIA LYSATE AND METHODS OF MAKING AND USING THEREOF	

DECLARATION OF JONATHAN J. BERMAN

I, Jonathan J. Berman, reside at 6205 Poindexter Lane, Rockville, MD 20852, declare the following:

1. I have a Ph.D in physics and an M.D. My curriculum vitae is attached.
2. I am the Director, Office of Clinical and Regulatory Affairs, National Center For Complementary and Alternative Medicine of the National Institutes of Health.
3. I have extensive experience in clinical evaluation and drug development with a specialized focus on *Leishmaniasis* and malaria.
4. I have reviewed and understand the Office action mailed 24 February 2004 in the above-referenced application.
5. I have reviewed and understand the pending claims in the above-referenced application.
6. I have reviewed and understand the prior art cited in the Office action, which the cited prior art is:
   a. Leishmania Research project DoD-8B, entitled "Infections *Leishmaniasis* Project Summary". Copy attached.
   b. Stitler et al. (1994) "Good Manufacturing Practices (GMP) Production of *Leishmania* Skin Test Antigen: 1. Protocol Requirements for Investigative New Drug (IND) Application" 44rd Annual Meeting of the American Society of Tropical Medicine and Hygiene. Abstract 179. Copy attached.
   c. Stitler et al. (1994) "Good Manufacturing Practices (GMP) Production of *Leishmania* Skin Test Antigen: 2. Production of a Microfluidized Lysate (MFL) LSTA" 44rd Annual Meeting of the American Society of Tropical Medicine and Hygiene. Abstract 179. Copy attached.
7. Leishmania Research project DoD-8B does not disclose that:
   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

8. Stitler et al. (1994) does not disclose that:

   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

9. Stitler et al. (199s) does not disclose that:

   a. The preparations are free of dextran.
   b. The preparations were microfluidized by a sudden release of pressure.
   c. The preparations contain phenol.

10. Simply reformulating a preparation in order to prevent hypersensitivity does not indicate that the preparation is free of dextran as there are many pharmaceutical preparations that contain dextran but do not cause hypersensitivity.

11. The indication that a preparation is a liquid product does not indicate that the preparation contains phenol as there are numerous solutions, solvents, buffers, and pharmaceutical carriers that are used for liquid formulations.

12. There are other ways to microfluidize a preparation which include freeze thawing and sonication. Thus, simply indicating that a preparation is microfluidized does not indicate the specific method by which the preparation was microfluidized.

13. In my opinion, the cited prior art references do not enable one skilled in the art to make and use the microfluidized leishmania lysate preparations of the above-referenced application. Specifically, the cited prior art does not teach microfluidized leishmania lysate preparations free of dextran and microfluidized by a sudden release of pressure. Further, the cited prior art does not teach the use of phenol in the preparations.

14. Further, in my opinion, it would not be obvious to one skilled in the art, such as myself, to remove dextran from the formulations in order to prevent hypersensitivity since there are many pharmaceutical preparations that contain dextran but do not cause hypersensitivity.

15. I declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

17 June 2004

Ret. Col. Jonathan Berman, MD, Ph.D.

Date

CURRICULUM VITAE--JONATHAN D BERMAN

1. VITAL INFORMATION

EDUCATION:

Jun 1967	B.A., cum laude, High Honors (Chem), Phi Beta Kappa: Williams College
Jan 1972	Ph.D., Biophysics: Harvard University.
Jun 1974	M.D.: Einstein School of Medicine.

2. BOARD CERTIFICATION/TRAINING

Diplomate, American Board of Pediatrics, February 1983.

3. BRIEF CHRONOLOGY OF EMPLOYMENT

1974-1976	Intern and Resident, Pediatrics, Mount Sinai Med Center, N Y.
1976-1977	Infectious Disease Fellow, Cornell Medical Center, New York.
1977-1980	Clinical Associate, Laboratory of Clinical Investigation, Laboratory of Parasitic Disease, NIAID, NIH, Bethesda, MD.
1980-1984	Parasitologist, Division of Experimental Therapeutics (DIV ET), Walter Reed Army Institute of Research (WRAIR) DC.
1984-1988	Clinical Director, Antileishmanial Drug Program, DIV ET
1984-1988	Chief, Biology Department, DIV ET
1988-1989	Assistant Director, Plans and Overseas Operations, WRAIR.
1989-1992	Associate Director, Plans, WRAIR.
1990-1994	Head, AIDS Opportunistic Infections, WRAIR.
1992-2002	Executive Officer, DIV ET
1992-2002	Chief, Biology Department, DIV ET
1999-2002	Research Coordinator: Malaria Drug Discovery and Development
2001-2002	Manager: Severe Malaria Drug Development
July 02 –pres	Dir, Office Clinical and Regulatory Affairs, NCCAM, NIH

4. MILITARY SERVICE

1977-1980	Public Health Service, Bethesda, MD.
1980-2002	U.S. Army Medical Corps-- COL (June 1989)
Aug 2002	Retired after 30 years of total service

5. COMMITTEES

1986-1988	Steering Committee, Leishmaniasis Chemotherapy, TDR/WHO
1991-1994	Ex Officio Member, DAIDS, NIH, Opportunistic Infection Core Committee
1991-1997	Clinical Subcommittee, Integrated Chemotherapy, TDR/WHO
1998- pres	External Product Manager, Miltefosine PDT, TDR/WHO
1998- pres	Chair, CME committee, Am Soc Trop Med Hyg
2002-pres	Chair, Paromomycin PDT, TDR/WHO.

6. RESEARCH INTERESTS

Alternative Med:	Clinical Evaluation
Leishmaniasis:	Biochem Pharmacology/Drug Development/Clinical Investigation
Malaria:	Drug Development / Clinical Investigation

7. IND DIRECTOR (STUDIES SUBMITTED TO US FDA)

DRUG	INDICATION	CO-DEVELOPMENT PARTNER	CLINICAL PHASES
Pentostam	Leishmaniasis RX	Wellcome	Pre/I/II/III/IV
Ketoconazole	Leishmaniasis RX	Janssen	II
Paromomycin	Leishmaniasis RX	Teva	Pre/I/II
WR 6026	Leishmaniasis RX	SKB	II
Pentamidine	Leishmaniasis RX	[none]	IV
Azithromycin	Malaria prophylaxis	Pfizer	II/III
WR 6026	P. carinii RX in HIV	NIAID, NIH	I
Azithromycin	M. avium proph in HIV	Pfizer	III

8. MANAGEMENT EXPERIENCE

Organizer/Director of large-scale, multicenter drug trials:

USA:	azithromycin for M. avium
Overseas:	antileishmanial and antimalarial agents

Contact with government/international agencies: FDA, NIH, DoD, WHO

Supervisor of 17-person Department.

Executive Officer for 100-person Division.

Director, Office Clinical and Regulatory Affairs NCCAM, NIH

9. PUBLICATIONS/PRIZES SUMMARIZED

Journal articles:	approximately 100
Review articles:	approximately 15

1997 Louis Weinstein award:Best Infectious Disease article in "Clinical Infectious Diseases" [1997; 24: 686-703]

Editorial Board: Antimicrobial Agents Chemotherapy (1998-2003)

Phi Beta Kappa: Williams College (1967)

"A" Proficiency Designator, USA Medical Corps, Sep 1997

NIH Grant recipient (# UC 1 A149500-01): Azithromcyin combinations for the treatment of P falciparum malaria (Co-PI)

10. MAJOR PUBLICATIONS (by number: R = review)

1) Berman JD, Young DM. Purification and properties of acetylcholinesterase. **Proceedings National Academy Science** USA 1971; 68: 395-398.

R4) Berman JD. Leishmaniasis Chemotherapy: biochemical mechanisms, clinical efficacy, and future strategies. **Reviews Infectious Diseases** 1988; 10: 560-586.

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Infections
Leishmaniasis
Project Summary

Title: Development of a Leishmania Skin Test Antigen (LSTA)

Synopsis: This study continues development of a skin test for leishmaniasis (like the skin test for tuberculosis) that would help diagnose this parasitic infection in Gulf War veterans and others who may have been exposed.

Overall Project Objective: Develop an intradermal skin test for the screening of U.S. Service members who may have been exposed to Leishmania parasites during deployments to leishmaniasis endemic areas.

Status/Results to Date: As reported last year, the lyophilized LSTA was reformulated into a liquid product to avoid a suspected hypersensitivity to a component of the lyophilization buffer. A new IND for this reformulated liquid Microfluidized-lysate (MFL)-LSTA was submitted to the FDA in 1999. A Phase I clinical trial was conducted in 15 healthy volunteers which demonstrated safety of the product by showing no significant local or systemic reactions to the product. Additionally, the product was administered in increasing dose and demonstrated that the skin test antigen had no significant local or systemic side effects when used at the planned maximal dose. A RFP was released to identify a commercial manufacturer for the future licensure of the LSTA product. A contract was awarded and phase I/II dose ranging and potency trials are underway.

Project:	DoD-8B
Agency:	Department Of Defense
Location:	Walter Reed Army Institute of Research
P.I. Name:	D. Scott Doughty
Research Type:	Development
Research Focus:	Leishmaniasis
Focus Category:	Infections
Status:	Ongoing
Study Start Date:	October 01,1993
Estimated Completion Date:	January 31,1999

Specific Aims: The goal is to identify a safe, potent, and non-sensitizing Leishmania Skin Test Antigen (LSTA); manufacture it under cGMP; obtain an IND for its use in phase I, II, and III clinical trials; and obtain ultimately a commercially available, FDA-licensed product.

available, FDA-licensed product.

Methodology: Skin tests are widely accepted diagnostic interventions for diagnosis of prior infection with an infectious agent (e.g., tuberculosis). Currently there is no Leishmania skin test licensed for use in the USA. Once required phase I and phase II studies are completed in humans, studies could be performed in Gulf War veterans with confirmed and suspected leishmaniasis.

Most Recent Publications:

None to date.

China University of Medical Sciences, Chengdu, P.R. of China; and General Hospital of Xinjing Petroleum Bureau, Karamay, P.R. of China.

fter sequencing the cloned kDNA fragments of the recombinant plasmid pLK 2, we have designed a set f oligomeric DNA primers (I and II) which defined 297 bp kDNA fragments. Dot hybridization analysis evealed it has species specificity. The minimal template kDNA detected is as low as 1 fg, and 2 romastigotes/ml. Amplifying the kDNAs from *Leishmania donovani* Sichuan human isolate, Sichuan anine isolate, *L. infantum*, *L. mexicana*, *L. braziliensis*, *L. major*, lizard *Leishmania*, positive products can be isualized only in *L. donovani* isolates and *L. infantum*. Dot hybridization of the amplified products with LK2 confirmed that they were *Leishmania* sequences. Based on this set of primers, 8 bone marrow and 4 erum samples from the confirmed visceral leishmaniasis patients were examined, 7 and 2 positive espectively. This result was also confirmed by Southern hybridization. It was shown in experimentally nfected golden hamsters that *L. donovani* kDNA could be detected as early as 4 days after infection, so arly diagnosis based on detecting kDNA in peripheral blood by PCR amplification is highly promising. Sequence homologies in kDNA of *Leishmania* species causing cutaneous leishmaniasis (CL) in Karamay, Xinjing were analyzed by PCR and kDNA hybridation. Specimens from cutaneous lesions of 8 CL patients 9 samples) were examined by PCR (using primer 13A, 13B), and the amplified products were hybridized with probes of *L. tropica* and *L. gerbilli* separately. Six samples (6/9) showed positive results with *L. tropica* and no hybridation (0/9) occurred with *L. gerbilli*. Southern hybridization was in accordance with those of dot hybridation. Our results suggest that homologous sequences exist within kDNA of *L. tropica* and the species causing CL in Karamay.

179 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN: 1. PROTOCOL REQUIREMENTS FOR INVESTIGATIONAL NEW DRUG (IND) APPLICATION. Stiteler JM*, Ballou WR, Eckels KH, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Institute of Research, Washington, DC.

Viscerotropic Leishmaniasis caused by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis in U.S. troops returning from Operation Desert Storm (ODS). The prevalence of *Leishmania* infection in ODS veterans is unknown. To determine the scope of infection in ODS veterans, a sensitive screening test is needed. One approach is to develop a *Leishmania* Skin Test (LST), which will meet FDA requirements for safety and efficacy. The first step in the development of a safe LST is the production of LST antigen (LSTA) under the strict conditions of the FDA's Good Manufacturing Practices (GMP). Compliance with GMP in production of the LSTA should allow for the approval of Human Use studies with the LSTA by the FDA following their review of an Investi- gational New Drug (IND) Application. Strain WR#1063, which was isolated from a bone marrow aspirate biopsy of a case of viscero- tropic Leishmaniasis was chosen as the type strain of *L.tropica* and source of the LSTA. WR#1063 was cloned, characterized as *L.tropica* by isoenzyme analysis, and then expanded and cryo- preserved as a Master Seed Lot (MSL). One sample of the MSL was expanded under conditions of GMP in WRAIR's Pilot Bioproduction Facility to produce a Production Seed Lot (PSL). Individual samples of the PSL were expanded under GMP to produce Bulk Lot Productions (BLP) of whole promastigotes for use in development of LST protocols for both animal as well as Human Use studies.

180 IDENTIFICATION OF A *TRYPANOSOMA CRUZI* RECOMBINANT ANTIGEN RECOGNIZED BY *T. CRUZI* INFECTED HUMANS AND MICE. Yong TS*, Minning TA, Khimani A, and Dusanic DG. Department of Life Sciences, Indiana State University, Terre Haute, IN.

A *Trypanosoma cruzi* antigen gene with diagnostic potential was identified by screening a Lambda ZAP cDNA library of epimastigote/metacyclic trypomastigotes of *T. cruzi* with laboratory infected BALB/c mice sera. The molecular weight of the fusion protein including β-galactosidase was 34 kDa. Western blots using epimastigote antigen and mice sera immunized with fusion protein showed two bands; 30 kDa and 27 kDa. The recombinant fusion protein reacted strongly with acutely and chronically infected mice and

human sera. Sixteen out of 20 (80%) protein by Western blot or ELISA. S leishmaniasis showed no reactivity recombinant protein. Data from So The insert was about 850 bp in leng

181 DIAGNOSIS OF SYMPTOM THE POLYMERASE CHAI Grogl M, and Berman J. D Research, Washington, DC; India; Federal University o Redwood City, CA; and Be

To diagnose symptomatic viscera aspirates, a polymerase chain rea Leishmania-infected macrophage parasitologically proven kala-aza sensitivity). None of 40 clinically of 13 clinically cured Indian pati (92%). This PCR procedure is ca before therapy, may identify pat substantially obviate the need fo

182 ENZYME POLYMORPH *BRAZILIENSIS*. Kreutz OH.

In a recent report which includ parasites isolated from Southw widely distributed isolates of e study over 200 isolates of L. (V to 20 enzymes) have been com species. Few of the enzymes w polymorphism appears to be patients with mucocutaneous frequency comparisons amon Belize), and the MCL enzyme Isolates of this New World sp MPI, and 6PGDH.

183 ANTIBODY TO TRYP Gabourel I, Bryan J*, Ministry of Health, B the Health Sciences, I

A study was conducted to d disease among three popul Force and from workers on enzyme-linked immunoass radioimmunoprecipitation City Hospital were reactiv

Gushulak B, Gully P, and Blajchman M. Faculty of Medicine - M.D. Programme, McMaster University, Hamilton, ON, Canada; Parasitology, St.Joseph's Hospital and Pathology, McMaster University, Hamilton, ON, Canada; Quarantine Health Services, Health Protection Branch, Health Canada, Ottawa, ON, Canada; and Canadian Red Cross Society and Haematology & Pathology, McMaster University, Hamilton, ON, Canada.

Our goal was to design a culturally acceptable study which will provide a valid estimate of the sero-prevalence of *Trypanosoma cruzi* in Latin-American refugees and immigrants to Canada. A literature search was undertaken to: a) review the scientific research available on *T. cruzi* parasitemia in Canada and the United States, b) explore the current interaction between the Latin-American community in the study area and the Canadian health care system, and c) identify the health programs which are currently in place to service the Latin-American community in the study area. Collaboration with health care workers within the Latin-American community was sought. The implications of the study for the Latin-American community were identified and suitable methods to undertake the study in a culturally-sensitive manner were formulated. We determined a sample size of 450 will be needed to be 95% confident of a sero-prevalence of 5% (plus or minus 2%). These samples will be tested by immunoflourescence or ELISA. A demographic data sheet was developed to stratify participants according to risk factors for antibodies to *T. cruzi*. Barriers to satisfactory interaction of the Latin-American community with the health care system were identified. Recommendations were formulated to ensure the greatest benefit of the study to the Latin-American community. These recommendations addressed the following four issues: 1) community education 2) information dissemination and informed consent 3) follow-up and management. 4) anonymity and confidentiality. printed in Spanish and in Portuguese, as well as English. 3) A clear management plan will be offered to identified participants who test positive for *T. cruzi* including referral to a tropical disease clinic and long-term follow-up. 4) Participants will be given anonymity unless they choose otherwise. All test results will remain confidential.

299 THE DRUG SENSITIVITY PROFILE OF FREE AMASTIGOTES: DEVELOPMENT OF A NEW MODEL SYSTEM FOR SCREENING DRUGS. Grogl M, Portal AC, and Callahan HL. U.S.A. Medical Research Unit-Brazil, Walter Reed Army Institute of Research.

Recently, there have been increasing reports in the literature of at least partially successful *in vitro* culture of "free" amastigotes. Similarly to a drug screen using promastigotes, a drug screen using free amastigotes should be relatively quick and easy, but should be more representative of the situation *in vivo*. In addition, it should alleviate the problems associated with testing drugs against amastigotes in macrophages. We have established an amastigote drug screen using free amastigotes from an L. mexicana (M379) strain as described previously. A comparison of the IC_{50} drug sensitivity profiles of the promastigote and amastigote stages of M379 against reference antileishmanials shows amastigotes and promastigotes respond equally to 3 out of 5 drugs tested. For the other 2 drugs, the IC_{50}s of the free amastigotes are more similar to values found testing amastigotes in macrophages than are the values found testing promastigotes. As expected, amastigotes were more sensitive than promastigotes to all antimony compounds tested (nearly 4-fold to 280-fold depending on the source). A comparison with achievable serum levels *in vivo* (where known) will also be presented.

300 GOOD MANUFACTURING PRACTICES (GMP) PRODUCTION OF *LEISHMANIA* SKIN TEST ANTIGEN (LSTA): 2. PRODUCTION OF A MICROFLUIDIZED LYSATE (MFL) LSTA. Stiteler JM*, Ballou WR, Eckels KH, Wellde BT, Topper MJ, Rowton ED, and Magill AJ. Division of Communicable Diseases & Immunology, Walter Reed Army Instituteof Research, Washington, DC.

Viscerotropic Leishmaniasis (VTL) resulting from infection by *Leishmania tropica* was described as a new clinical presentation of Leishmaniasis following isolation and characterization of the parasite from U.S. troops returning from Operation Desert Storm (ODS). The prevalence of VTL in ODS veterans is unknown. The USA/DoD decided to pursue the development of a LSTA for use as such a diagnostic screening method to determine exposure of personnel to *L. tropica*. A soluble, lyophilized, Microfluidized lysate (MFL) LSTA was developed and produced in accordance with FDA's guidelines for current GMP within WRAIR's Pilot Bioproduction Facility. Strain WR#1063 which was isolated from a bone marrow aspirate biopsy of a case of VTL was chosen as the type strain and source of the MFL-LSTA. WR#1063 was cloned, characterized, and then expanded and cryopreserved (MSL). One sample of the MSL was then expanded (PSL). Individual cryostocks of the PSL of WR#1063 promastigotes were grown, harvested, washed, and stored (BLP). Various BLP processing experiments and animal testing of these LSTA preparations led to the current MFL-LSTA protocol. In brief, the BLP was thawed, microfluidized, centrifuged, the supernatant sterile filtered, the filtrate adjusted to dose, lyophilized as MFL-LSTA. Following required testing of the MFL-LSTA, an INDA was prepared for review by FDA. FDA's approval of human use will lead to Phase I/Phase II trials of the LSTA.